

Synthesis and study of nanoensemble, based on graphene oxide conjugated with quercetin-containing extract, extracted from onion peel (*Allium cepa* L.)

Z.O. Gakhramanova¹, S.S. Hasanova^{1,3}, N.H. Mamedova², A.A. Israyilova³, G.S. Aliyeva³, T.O. Rahimov², T.C. Ismailova², G.S. Kerimova²

¹Scientific Research Institute “Geotechnological Problems of Oil, Gas and Chemistry”, Ministry of Science and Education of Azerbaijan, Dilara Aliyeva Street 227, AZ1010 Baku, Azerbaijan

²Azerbaijan Medical University, Samad Vurgun Street 155, AZ1022 Baku, Azerbaijan

³Baku State University, Zahid Khalilov Street 23, AZ1148 Baku, Azerbaijan

Abstract

This study presents investigation of newly obtained antibacterial agents synthesized through functionalization of graphene oxide with quercetin-containing extract from the onion (*Allium cepa*), and subsequent antibacterial analysis of the resulting nanoensemble. Quercetin containing extract been investigated by HPLC. The structure and morphology of the resulting nano ensemble was confirmed by FTIR spectroscopy, powder X-ray diffraction, and Transmission Electron Microscopy.

Key words: graphene oxide, quercetin, nanoensemble, antibacterial activity.

*Corresponding author. Tel. +994554807033

E-mail address: zarema_q@bk.ru (Z. Gakhramanova)

1. Introduction

The increase in the number of infections and the significant growth of resistance of microorganisms to antibiotics are among the crucial problem the world health care system is facing. Formation of resistant strains of bacteria that significantly complicate treatment, are recognized as a very important global socio-medical problem. Thus, it seems particularly relevant to search for natural compounds that can be used as an alternative supplement to antibiotics. In this aspect, scientists mind are attracted by the bioflavonoids that are the natural phytochemical substance containing polyphenolic structure. It is famous for its antioxidant, anti-inflammatory, and antimicrobial properties [1]. It is widely distributed in various fruits, vegetables, drinks, as well as in flowers, leaves, seeds, etc. The most widespread of the bioflavonoids is quercetin that demonstrate wide range of biologically active properties. The most abundant source of quercetin is onion (*Allium cepa*), which is one of the most popular vegetables used both as an edible and medicinal plant. Quercetin is one of the common flavonoids that demonstrate antimicrobial activity, effectively inhibiting growth of various bacteria [2,3,4].

However, quercetin's poor water solubility doesn't allow to cell membranes effectively adsorb this compound, that is why there is interest in improving these shortcomings by increasing its bioavailability [4].

In turn, the use of modern nanomaterials opens up great prospects for their use for biomedical purposes. Nanomaterials based on graphene and its derivatives have received wide interest due to their unique

physicochemical properties. Graphene Oxide (GO) has attracted much attention in biomedical applications due to its biocompatibility and promising properties such as high specific surface area with lots of oxygen containing functional groups (epoxy, hydroxy and carboxy) that allow effectively functionalize its surface with molecule of interest, to bring the novel and useful properties such as solubility, biological activity, mechanical strength, thermal stability, optical properties etc. [5]. Over the past few years, GO has been found to have excellent immobilization ability for many substances including metals, drugs, biomolecules, and fluorescent molecules with anticancer, antibacterial, and antiviral properties [6,7,8].

Considering all we mentioned above, we carried out the synthesis of new nanoensemble based on graphene oxide (GO) with quercetin-containing extract. The obtained nanostructure have been investigated and analyzed for biological activity.

2. Experimental part

Reagents and materials. Ethanol (70%), ethyl acetate, graphite, NaNO_3 , H_2SO_4 , KMnO_4 , distilled water, H_2O_2 (3%), DMSO-d6 have been purchased from Sigma Adrich and used without further purification.

Extraction. Extraction of quercetin. Onion wastes were selected as the raw material for extract quercetin-containing extracts. Initially, the bunch of onion peel was rinsed under a stream of distilled cold water and dried at room temperature. After that the cleaned raw material was ground to a size of 3–5 mm. Subsequently 50 grams of the crushed wastes were soaked in 300 mL of ethyl acetate and maintained in a water bath at 60–70° for 3–4 hours applying reflux condenser. The resulting onion husk solution was filtered and dried in a thermostat at the temperature of 40–50°C. Obtained powder was recrystallized by using 70% ethanol, yielding 0.2 grams.

Preparation of quercetin containing extract. In the case of obtaining the onion husk extract, 70% ethanol was used as the solvent, and drying was carried out using a rotary evaporator with a vacuum pump of extract. The obtained product was the light yellow powder.

Synthesis of graphene oxide. Graphene oxide was prepared by a Hummers method, a method that is renowned for increasing the efficiency, safety, and general quality of production of GO, thereby ideal for both research and industrial purposes.

In short, 10 g of graphite and 5 g of sodium nitrate were dispersed in 250 mL of sulfuric acid and were stirred for 15 min while maintained in ice bath. Thereafter, potassium permanganate was slowly added to the reaction mixture with control to keep it below 20 °C. Upon addition of this, the reaction mixture was stirred for 5 h while gradually raising the temperature to 35 °C.

The oxidation process was followed by quenching with the addition of 450 mL of deionized water. Next, 2500 mL of hydrogen peroxide solution in the form of 3% hydrogen peroxide was introduced to solution. The product was centrifuged and rinsed with distilled water thoroughly under conditions where the pH became neutral.

Synthesis of GO@Quercetin containing extract. 0.05 g of graphene oxide nanoparticles in 15 mL of DMSO was dispersed in ultrasonic bath for 15 minutes. Then, 0.15 g of the active compound (quercetin) was slowly added to the graphene oxide nanoparticle mixture, followed by continuous stirring for 48 hours.

Characterization of graphene oxide and obtained nanoensemble

FTIR spectroscopy. The synthesized structures- the quercetin extracted from the onion and graphene oxide have been investigated by FTIR analysis. The spectra were acquired using a Thermo™Scientific™ iS FTIR spectrophotometer. FTIR analysis was conducted to investigate the molecular structure and identify the functional groups present in the materials.

X-Ray powder diffraction analysis (PXRD). X-Ray diffraction (XRD) analysis was conducted under ambient conditions using a Rigaku MinFlex 600 diffractometer to elucidate the crystalline phases and structural features of the synthesized graphene oxide

Transmission electron microscopy (TEM). The TEM analysis of the graphene oxide and GO@Quercetin containing extract was investigated on a TEM JEOL-1400 (Japan) at 80–120kV.

HPLC. The obtained extract and quercetin were qualitatively analyzed and compared with the standard solution of quercetin by high-performance liquid chromatography with diode array (HPLC-DMD).

Biological analysis. The antibacterial activity of the investigated compounds against bacteria *E. coli* and *S. aureus* was assessed determining minimum inhibitory concentration (MIC) by twofold microdilution method.

Determination of minimum inhibitory concentration. The minimum inhibitory concentration of samples (Quercetin, GO, PE (Quercetin containing extract), GO@PE, GO@Quercetin) was determined by resazurin dye assay [1-4] on clinical isolates *E. coli* and *S. aureus* in accordance with CLSI guidelines. The yeast cultures used in the microbiological studies were taken from the culture collection of Baku State University (Azerbaijan). The compounds were prepared in DMSO according to the CLSI standards and diluted in 96 well microtiter plates in Muller Hinton Broth (MHB) ("Liofilchem"). The concentration of the assessed substances ranged from 256 to 2 µg/mL, afterwards fungal strains were inoculated into each well and plates were incubated for 18 hours at 37°C. The final density of the test cultures was adjusted to 0.5 McFarland by using a digital densitometer. Xmark (Bio-Rad) plate reader was used for the reading of the experimental plates before (0 h) and after incubation (18h). In addition to this 30 µL of resazurin dye (0.01%) (Sigma Aldrich) was added to each well and microplates were again placed in an incubator for 3-4 hours. The colour change from blue to pink is considered as evidence of bacterial growth. This way the minimum inhibitory concentration (MIC) is considered as the concentration after which the tested compounds do not inhibit this change in colour. Gentamicin and Ciprofloxacin were used as a control for *S. aureus* and *E. coli*, respectively

Growth inhibition. Growth inhibition assay were performed according to the guidelines by CLSI. Bacterial strains were stored on Tryptic Soy Agar (TSA) at 37°C for 18 h. The wells were treated with the samples and concentration of the compound was ranged between 256 – 2 µg/mL. After treatment, 96 well plates were incubated at 37°C for 18 h. Xmark (Bio-Rad) plate reader was used for the reading of

the experimental plates before (0 h) and after incubation (18 h). The growth inhibition percentage was calculated relative to vehicle control (DMSO). The MIC was determined as the lowest concentration with $\geq 90\%$ inhibition. Test were repeated in duplicate with three technical replicates per treatment and on different days. The graphs were prepared on Graphpad Prism 10.0 Software

3. Results and discussion

Quercetin is a natural flavonoid with a number of biologically active properties, however, the low solubility of quercetin in water does not allow cell membranes to effectively adsorb this compound, which opens up great prospects for modifying it with various nanomaterials. Various modifications were carried out with nanoparticles such as: quercetin was modified with mesoporous silica nanoparticles [9], polymeric nanoparticles [10], chitosan-alginate nanoparticles [11], chitosan tripolyphosphate nanoparticles [12]. The non-covalent functionalization of GO with quercetin from the onion (*Allium cepa*), have not been presented and tested against *E. coli* and *S. aureus* yet. In view of the above, we have functionalized graphene oxide with quercetin containing extract. Obtaining of GO been prepared by Hummers method [5]. The reaction scheme is presented below.

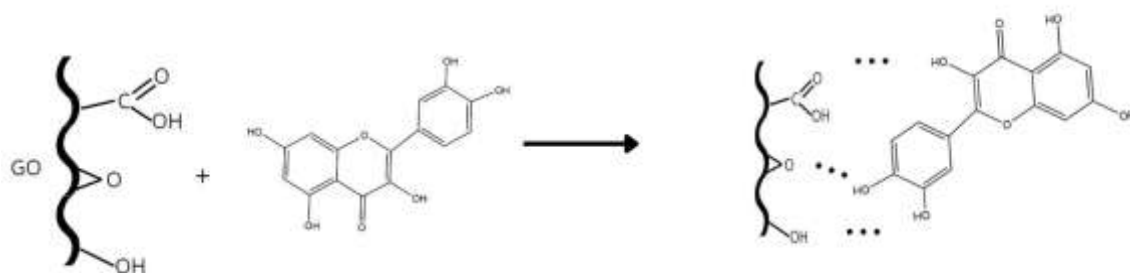


Figure 1. Synthesis of nanoensemble

The obtained extract, GO and nanoensemble have been analyzed by FTIR, XRD, HPLC methods. The morphology of prepared nanostructure was investigated by transmission electron microscopy.

FTIR spectra analysis was performed to investigate the structure and functional groups of the prepared materials. FTIR analysis of GO showed O-H ($\sim 3406\text{ cm}^{-1}$), C-H,CH₂ ($\sim 2959\text{ cm}^{-1}$), C=O ($\sim 1722\text{ cm}^{-1}$), C-O ($\sim 1177\text{ cm}^{-1}$), peaks, indicating successful oxidation of graphite flakes. FTIR analysis of GO@Quercetin nanoensemble reveals decrease of the absorption band of the hydroxyl group to 3270 cm^{-1} that indicates the formation of hydrogen bonds during functionalization.

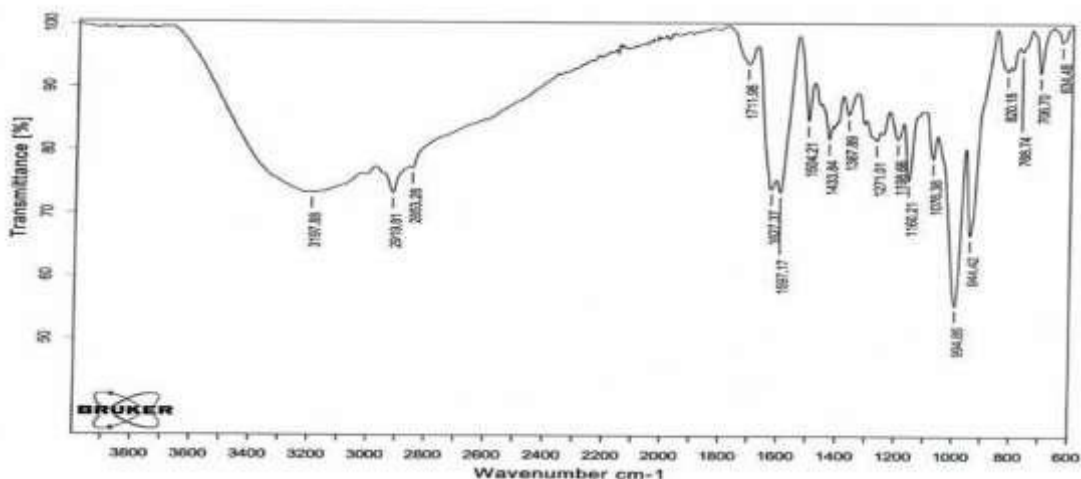


Figure 2. FTIR-spectrum of GO@Quercetin nanoensemble

XRD peaks position reveals composition, while the intensity provides structural and phase information. GO demonstrate exhibits characteristic peaks in Fig. 3 it is $2\theta \sim 11^\circ$ and is $2\theta \sim 42^\circ$ showing the distance between layers of graphite after oxidizing of graphite.

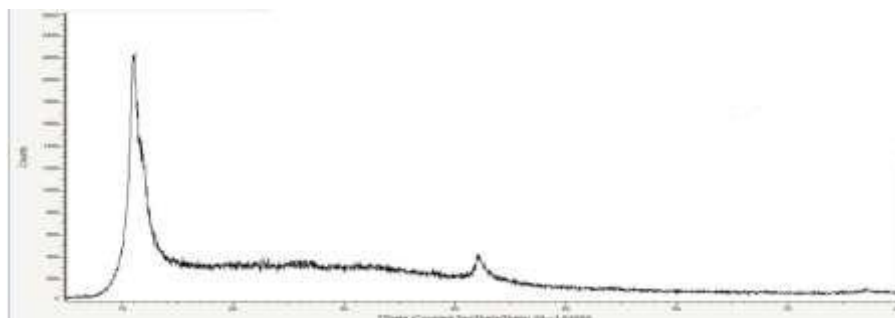


Figure 3. XRD pattern of graphene oxide.

The morphology of graphene oxide in Fig. 4(a) is shown as GO sheets consisting of several layers stacked on top of each other, which ensures minimal creasing.

In the case of TEM images of GO conjugated with quercetin and crude extract demonstrated in Fig 4(b,c), the strong compaction on the dark areas shows the formation of hydrogen bonds in the obtained substances.



Figure 4 (a). TEM image of GO



Figure 4 (b). TEM image of GO@Quercetin

Figure 4 (c). TEM image of GO@crude extract

HPLC. For the identification of quercetin, a wavelength equal to 368 and 254 nm was chosen, as mobile phase 1.5% acetic acid and water/acetonitrile/methanol ratio 55:40:5.

As a result the HPLC analysis reveal that identical peaks were detected, indicating the presence of quercetin in both investigated substances.

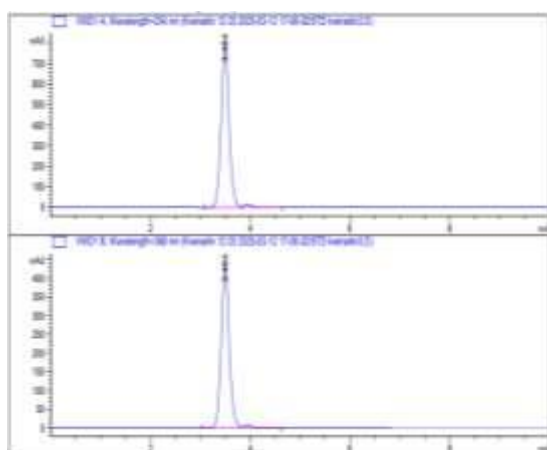


Figure 5 (a). HPLC analysis of standard (PE) quercetin solution.

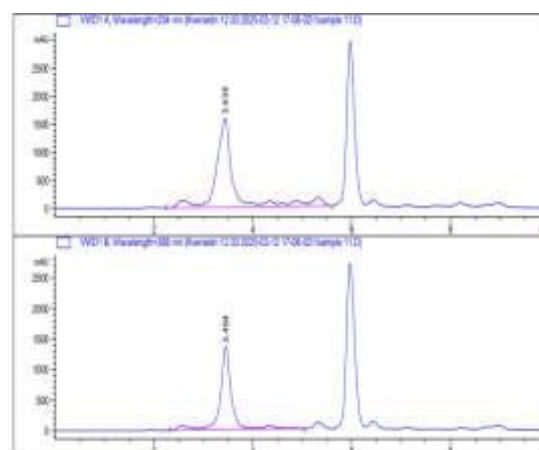


Figure 5 (b). HPLC analysis of crude plant extract

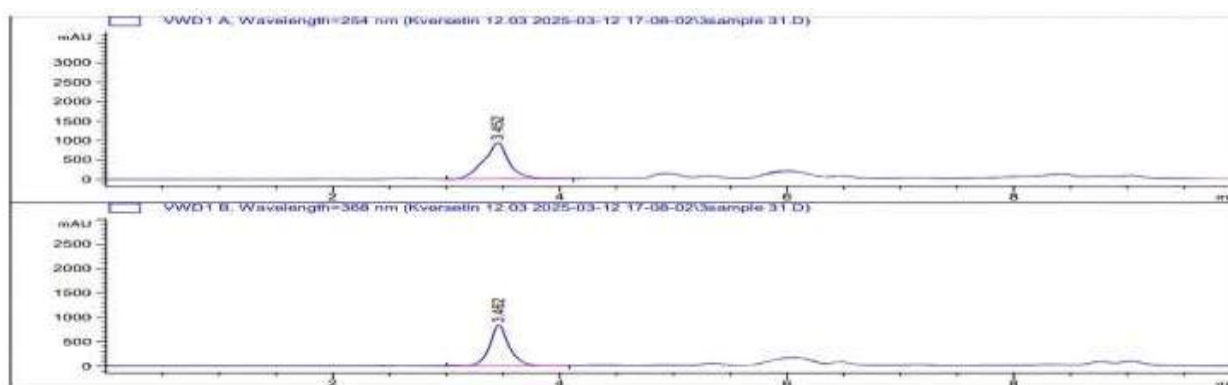


Figure 5 (c). HPLC analysis of quercetin extracted from the onion (*Allium cepa*),

The antimicrobial activity of the tested compounds against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was evaluated by determining their minimum inhibitory concentrations (MICs).

The Quercetin containing crude- plant extract (PE) exhibited moderate antibacterial activity, with MIC values of 8 $\mu\text{g/mL}$ against *E. coli* and 16 $\mu\text{g/mL}$ against *S. aureus*. It was revealed that, the GO@PE composite showed a significantly enhanced antibacterial effect, with MIC values reduced to 2 $\mu\text{g/mL}$ and 4 $\mu\text{g/mL}$ against *E. coli* and *S. aureus*, respectively. This suggests a potential synergistic effect between GO and PE when combined, resulting in improved antimicrobial potency.

In contrast, GO alone demonstrated poor antimicrobial activity, with high MIC values of 256 $\mu\text{g/mL}$ for both bacterial strains, indicating limited efficacy when used individually.

The positive control antibiotics also demonstrated potent antibacterial activity. Ciprofloxacin (CIP) had an MIC of 2 $\mu\text{g/mL}$ against *E. coli* and Gentamicin (GEN) inhibited *S. aureus* with an MIC of 2 $\mu\text{g/mL}$.

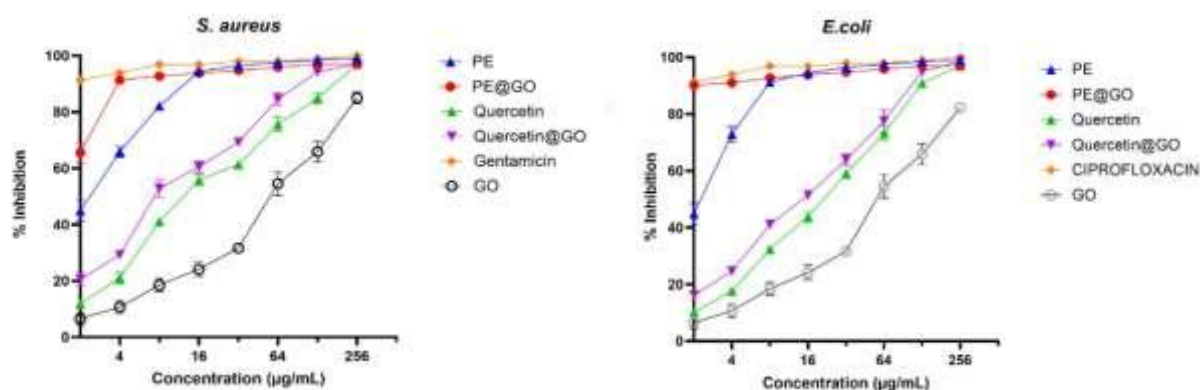


Figure 6. Growth inhibition of *S. aureus* and *E. coli*

4. Conclusion

We successfully isolated quercetin-containing extract from the onion (*Allium cepa*), and then successfully functionalized it with graphene oxide. The morphology and structure were confirmed by using physicochemical methods of analysis. Biologically active compounds were tested for antibacterial activity. While quercetin alone showed limited antibacterial activity against both bacterial strains, its conjugated form with graphene oxide led to significant increase of antibacterial activity. In conclusion, the results obtained emphasize the higher efficacy of GO@PE composite compared to its individual components and indicate its potential as a promising antimicrobial agent, especially against both Gram-negative and Gram-positive bacterial strains.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research.

References

1. Yi, H., Peng, H., Wu, X., Xu, X., Kuang, T., Zhang, J., Du, L., and Fan, G. The therapeutic effects and mechanisms of quercetin on metabolic diseases: pharmacological data and clinical evidence. *Oxidative medicine and Cellular Longevity*. 2021. <https://doi.org/10.1155/2021/6678662>
2. Veiko, A.G., Olchowik-Grabarek, E., Sekowski, S., Roszkowska, A., Lapshina, E.A., Dobrzynska, I., Zamaraeva, M., Zavodnik, A.B. Antimicrobial activity of quercetin, naringenin and catechin: flavonoids inhibit staphylococcus aureus-induced hemolysis and modify membranes of bacteria and erythrocytes. *Food Bioactive Compounds: Chemical Challenges and Opportunities*. 2023. <https://doi.org/10.3390/molecules28031252>.
3. Nguyen, T.L.A., and Bhattachary, D. Antimicrobial activity of quercetin: an approach to its mechanistic principle. *quercetin: From Structure to Health*. 2022. <https://doi.org/10.3390/molecules27082494>
4. Batiha, G.E.S., Beshbishy, A.M., Ikram, M. The Pharmacological Activity, Biochemical Properties, and Pharmacokinetics of the Major Natural Polyphenolic Flavonoid: Quercetin. *Plant Extracts: Chemical Composition, Bioactivity and Potential Applications*. 2020. <https://doi.org/10.3390/foods9030374>
5. Liu, J., Shuping, C., Yanan, L., Bijing, Z. Progress in preparation, characterization, surface functional modification of graphene oxide. *Journal of Saudi Chemical Society*. 2022. <https://doi.org/10.1016/j.jscs.2022.101560>
6. Tiwari, H., Karki, N., Pal, M. Functionalized graphene oxide as a nanocarrier for dual drug delivery applications: The synergistic effect of quercetin and gefitinib against ovarian cancer cells. *Colloids and Surfaces B: Biointerfaces*. 2019. <https://doi.org/10.1016/j.colsurfb.2019.03.037>
7. Jampilek, J., and K. Kralova, K. Materials, advances in drug delivery nanosystems using graphene-based materials and carbon nanotubes. *Functional Nanomaterials and Biopolymers for Precision Medicine*. 2021. <https://doi.org/10.3390/ma14051059>
8. Lee, J., Kim, J., Kim, S. Biosensors based on graphene oxide and its biomedical application. *Advanced Drug Delivery Reviews*. 2016. <https://doi.org/10.1016/j.addr.2016.06.001>
9. Sarkar, A., Ghosh, S., Chowdhury, S., Pandey, B. Parames C, Targeted delivery of quercetin loaded mesoporous silica nanoparticles to the breast cancer cells. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2016. <https://doi.org/10.1016/j.bbagen.2016.07.001>
10. Baksi, R., Singh, D.P., Borse S.P. In vitro and in vivo anticancer efficacy potential of Quercetin loaded polymeric nanoparticles. *Biomedicine & Pharmacotherapy*. 2018. <https://doi.org/10.1016/j.biopha.2018.07.106>
11. Aluani, D., Tzankova, V., Kondeva-Burdina, M., Yordanov, Y. Evaluation of biocompatibility and antioxidant efficiency of chitosan-alginate nanoparticles loaded with quercetin. *International Journal of Biological Macromolecules*. 2017. <https://doi.org/10.1016/j.ijbiomac.2017.05.062>
12. Choudhary, A., Kant, V., Jangir, B.L., Joshi, V.G. Quercetin loaded chitosan tripolyphosphate nanoparticles accelerated cutaneous wound healing in Wistar rats. *European Journal of Pharmacology*. 2020. <https://doi.org/10.1016/j.ejphar.2020.173172>